



COMMENTARY

Excitation–Contraction Coupling

Orai1 as a potential “fits-all approach” therapeutic target for the treatment of DMD

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Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by loss-of-function mutations in the dystrophin gene. DMD is a progressive disease that affects 1 in 3,500–5,000 male births and results in muscle weakness, respiratory and cardiac failure, and, in due course, premature death commonly in the third decade of life (Mendell and Lloyd-Puryear, 2013). Histopathological hallmarks of DMD muscles include central nucleation, fibrosis, inflammation, and muscle fiber pseudohypertrophy amongst others (Zweyer et al., 2022). The dystrophin gene was discovered in 1987 (Hoffman et al., 1987), and since then there have been numerous efforts to develop therapies to delay disease progression and enhance muscle function in DMD patients.

Present widespread therapeutic recommendations include corticosteroids, cardiac medications, and, eventually, assisted ventilation to slow down disease progression, reduce symptoms, and improve quality of life, but do not target the underlying mechanism of the disease. However, pharmacological advances using oligonucleotide-mediated exon skipping to bypass variant exons and gene editing to provide truncated forms of dystrophin have gained interest in recent years, and several clinical trials are under way to assess their efficacy (Eser and Topaloglu, 2022). Although these are promising advancements, there are still challenges to overcome and room for improvement in the area. For example, the TREAT-NMD DMD global database contains over 7,000 dystrophin mutations (Bladen et al., 2015), raising significant challenges for the development of gene correction therapies that might be applicable to large cohorts of patients rather than single individuals. Moreover, present exon-skipping therapies focusing on restoring dystrophin at low levels are suggested to have the potential to benefit ~30% of all patients (Eser and Topaloglu, 2022) and hence also struggle to treat a large cohort of DMD individuals. However, the largest challenge to the field of therapeutic genome editing might be the

high cost of these drugs (Segal, 2022). Nevertheless, there is still a need for curative treatments suitable for a large group of DMD individuals, and pharmacological interventions with a “fits-all approach” are desired. This is one of the reasons the recent publication by García-Castañeda et al. (2022) and colleagues in Dr. Robert T. Dirksen’s lab is attractive with its obvious transferability into drug development.

Chronically elevated levels of myoplasmic free $[Ca^{2+}]$ is an acknowledged explanation for the observed skeletal muscle fiber deterioration and loss-of-function in DMD. Two main pathomechanisms have been proposed to cause sustained abnormalities in myoplasmic Ca^{2+} in DMD (see Fig. 1); enhanced RyR1-mediated Ca^{2+} leak from the sarcoplasmic reticulum (SR; Bellinger et al., 2009) and excessive extracellular Ca^{2+} influx via membrane tears and/or store-operated Ca^{2+} entry (SOCE; Edwards et al., 2010; Zhao et al., 2012). The Ca^{2+} sensor stromal interaction molecule-1 (STIM1) in the ER/SR membrane and the highly Ca^{2+} -selective Orai channel in the plasma membrane forms the basis for SOCE (Emrich et al., 2022).

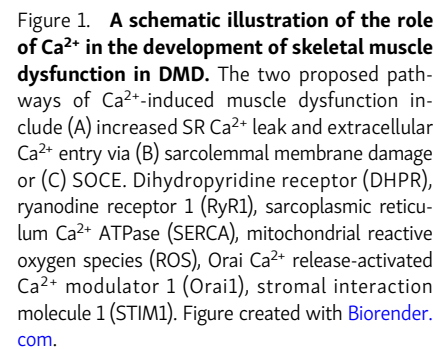
Here, García-Castañeda and colleagues used skeletal muscle-specific tamoxifen-inducible Orai1-knockout mice crossed with mdx mice to elucidate the impact of Orai1-dependent Ca^{2+} entry in the DMD muscle pathology (Fig. 1). This approach allowed them to knockout Orai1 in the skeletal muscle of young, post-developmental mice and thus establish the effects of inducing a change into Ca^{2+} entry after the disease manifestation. Excitingly, eliminating Orai1-mediated Ca^{2+} influx in young (2–3-mo-old) mdx mice resulted in considerable functional and morphological improvements. Skeletal muscle from mdx mice lacking Orai1-mediated Ca^{2+} influx exhibited normalized intracellular Ca^{2+} homeostasis and improved muscle strength. Obliterating Orai1 expression also had a protective effect on the plasma membrane (sarcolemma) in mdx mice as it protected muscles from eccentric contraction-induced damage. Further, muscles from mdx mice

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This work is part of a special issue on excitation–contraction coupling.

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clinically manifests as progressive muscle weakness, it is the respiratory failure and heart problems (often develop in the form of dilated cardiomyopathy) that ultimately causes the premature death. The diaphragm is a skeletal muscle with a similar cytoskeleton, extracellular matrix, and excitation-contraction (EC) coupling as the limb muscles examined by García-Castañeda and colleagues, and hence one could speculate that it is likely that the removal of Orail-mediated Ca^{2+} influx has beneficial effects in DMD muscle. On the other hand, there is more uncertainty whether eliminating Orail-mediated Ca^{2+} influx in cardiac muscle would have any effect given that cardiac muscle is designed to handle Ca^{2+} influx as its EC coupling, and thus every heartbeat, is initiated by Ca^{2+} influx via the voltage-gated Ca^{2+} channel (Cav2.1, L-type Ca^{2+} channel). Hopefully this and additional aspects of Orail physiology and pathophysiology in striated muscle will be addressed by the Dirksen laboratory or others in the near future. Nevertheless, the results of [García-Castañeda et al. \(2022\)](#) demonstrate an important role of enhanced Orail-mediated Ca^{2+} entry in exacerbating the dystrophic phenotype of mdx mice, rendering Orail a potential therapeutic target with a “fits-all approach” for the treatment of DMD. On that note, it is promising that there is a continuous development of Orail inhibitors ([Azimi et al., 2020](#)) for various indications, and some have entered clinical trials with DMD potentially included in the near future.

J.T. Lanner is supported by the Swedish Research Council.

Author contributions: A.J. Cheng, F. von Walden and J.T. Lanner drafted the manuscript and approved the final draft of the manuscript.

References

- Azimi, I., R.J. Stevenson, X. Zhang, A. Meizoso-Huesca, P. Xin, M. Johnson, J.U. Flanagan, S.B. Chalmers, R.E. Yoast, J.S. Kapure, et al. 2020. A new selective pharmacological enhancer of the Orail Ca^{2+} channel reveals roles for Orail in smooth and skeletal muscle functions. *ACS Pharmacol. Transl. Sci.* 3:135–147. <https://doi.org/10.1021/acsptsci.9b00081>
- Bellinger, A.M., S. Reiken, C. Carlson, M. Mongillo, X. Liu, L. Rothman, S. Matecki, A. Lacampagne, and A.R. Marks. 2009. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. *Nat. Med.* 15:325–330. <https://doi.org/10.1038/nm.1916>
- Berry, C.T., M.J. May, and B.D. Freedman. 2018. STIM- and Orail-mediated calcium entry controls NF- κ B activity and function in lymphocytes. *Cell Calcium*. 74:131–143. <https://doi.org/10.1016/j.ccca.2018.07.003>
- Bladen, C.L., D. Salgado, S. Monges, M.E. Foncuberta, K. Kekou, K. Kosma, H. Dawkins, L. Lamont, A.J. Roy, T. Chamova, et al. 2015. The TREAT-NMD DMD global database: Analysis of more than 7,000 Duchenne muscular dystrophy mutations. *Hum. Mutat.* 36:395–402. <https://doi.org/10.1002/humu.22758>
- Edwards, J.N., O. Friedrich, T.R. Cully, F. von Wegner, R.M. Murphy, and B.S. Launikonis. 2010. Upregulation of store-operated Ca^{2+} entry in dystrophic mdx mouse muscle. *Am. J. Physiol. Cell Physiol.* 299:C42–C50. <https://doi.org/10.1152/ajpcell.00524.2009>
- Emrich, S.M., R.E. Yoast, and M. Trebak. 2022. Physiological functions of CRAC channels. *Annu. Rev. Physiol.* 84:355–379. <https://doi.org/10.1146/annurev-physiol-052521-013426>
- Eser, G., and H. Topaloglu. 2022. Current outline of exon skipping trials in Duchenne muscular dystrophy. *Genes*. 13:1241. <https://doi.org/10.3390/genes13071241>
- García-Castañeda, M., A. Michelucci, N. Zhao, S. Malik, and R.T. Dirksen. 2022. Postdevelopmental knockout of Orail improves muscle pathology in a mouse model of Duchenne muscular dystrophy. *J. Gen. Physiol.* 154: e202213081. <https://doi.org/10.1085/jgp.202213081>
- Hoffman, E.P., R.H. Brown Jr, and L.M. Kunkel. 1987. Dystrophin: The protein product of the Duchenne muscular dystrophy locus. *Cell*. 51:919–928. [https://doi.org/10.1016/0092-8674\(87\)90579-4](https://doi.org/10.1016/0092-8674(87)90579-4)
- Kang, Q., X. Peng, X. Li, D. Hu, G. Wen, Z. Wei, and B. Yuan. 2021. Calcium channel protein ORAI1 mediates TGF- β induced epithelial-to-mesenchymal transition in colorectal cancer cells. *Front. Oncol.* 11:649476. <https://doi.org/10.3389/fonc.2021.649476>
- McCarl, C.A., C. Picard, S. Khalil, T. Kawasaki, J. Röther, A. Papolos, J. Kutok, C. Hivroz, F. Ledeist, K. Plogmann, et al. 2009. ORAI1 deficiency and lack of store-operated Ca^{2+} entry cause immunodeficiency, myopathy, and ectodermal dysplasia. *J. Allergy Clin. Immunol.* 124:1311–1318.e7. <https://doi.org/10.1016/j.jaci.2009.10.007>
- Mendell, J.R., and M. Lloyd-Puryear. 2013. Report of MDA muscle disease symposium on newborn screening for Duchenne muscular dystrophy. *Muscle Nerve*. 48:21–26. <https://doi.org/10.1002/mus.23810>
- Segal, D.J. 2022. The promise of gene editing: So close and yet so perilously far. *Front. Genome Ed.* 4:974798. <https://doi.org/10.3389/fgeed.2022.974798>
- Zhao, X., J.G. Moloughney, S. Zhang, S. Komazaki, and N. Weisleder. 2012. Orail mediates exacerbated Ca^{2+} entry in dystrophic skeletal muscle. *PLoS One*. 7:e49862. <https://doi.org/10.1371/journal.pone.0049862>
- Zweyer, M., H. Sabir, P. Dowling, S. Gargan, S. Murphy, D. Swandulla, and K. Ohlendieck. 2022. Histopathology of Duchenne muscular dystrophy in correlation with changes in proteomic biomarkers. *Histol. Histopathol.* 37:101–116. <https://doi.org/10.14670/hh-18-403>